Port Phillip Bay Environmental Management Plan: Monitoring the state of Bay nitrogen cycling (2002–2005)

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Port Phillip Bay Environmental Management Plan: Monitoring the state of Bay nitrogen cycling (2002-2005)

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Executive Summary

The Port Phillip Bay Environmental Management Plan recommended that a program be established to monitor the state of Bay nitrogen cycling. Coupled nitrification/denitrification (the microbial transformation of bio-available ammonium, nitrate and nitrite to inert nitrogen gas), plankton biomass, dissolved oxygen concentrations and dissolved organic nitrogen concentrations were all identified as sensitive indicators of water quality. The program now in place includes twice-yearly monitoring of denitrification at the sediment surface, continuous monitoring of some physicochemical characteristics, and collection of samples for dissolved organic nitrogen analysis as part of the EPA Fixed Sites program. This report covers the first three years of monitoring (August 2002-May 2005).

To determine denitrification efficiency, benthic nutrient fluxes were measured twice each year, in spring and autumn, in Hobsons Bay and central Port Phillip Bay. Continuous physicochemical monitoring has been carried out in bottom waters at three sites since August 2002, and in surface waters since March 2003. The sites are those from the EPA Fixed Sites monitoring program in the centre of Port Phillip Bay, in Hobsons Bay and at Long Reef near Werribee. Characteristics monitored include salinity, temperature, dissolved oxygen, chlorophyll fluorescence and Photosynthetically Active Radiation (PAR: light necessary for plant growth). Additional samples for dissolved organic nitrogen analysis have been collected by EPA on 10 occasions during 2002-2005.

All of the benthic nutrient fluxes were within the ranges previously measured in central Port Phillip Bay and Hobsons Bay. The data indicate generally higher fluxes at both sites in autumn than in spring, and much higher fluxes in Hobsons Bay in both seasons than in central Port Phillip Bay. Both of these observations also accord with past work, and reflect the generally higher supply of organic matter to the sediments in Hobsons Bay. The key indicator, denitrification efficiency, was always higher in central Port Phillip Bay than in Hobsons Bay. In Hobsons Bay in spring 2002, denitrification efficiency was higher than the historic median, which could be attributed to a lower than normal organic supply, due to low riverine inputs. However, carbon dioxide fluxes in later samples were closer to previous measurements, as was denitrification efficiency. There is some evidence of a fall in denitrification efficiency in autumn 2004 and autumn 2005 at both sites, compared to the previous spring. This is attributed to “patchiness” at the sample sites, rather than a long-term decline in bay health.

According to the PPBES model, a 30% decline baywide in denitrification efficiency would be reflected by a similar proportional increase in chlorophyll *a* concentration. No long-term statistical change has been detected for either indicator in the first three years of this program. At most, denitrification efficiency declined by ~ 20% at the Central site over 2004-05, and apart from the response to the flood in February 2005, chlorophyll *a* concentration has not increased over the period 2002-2005.

The indicators monitored here were also studied to determine the reponse to the Febriary 2005 floods in the Yarra and Werribee Rivers. The responses to the flood varied between sites, with short-lived responses in surface water chlorophyll at Hobsons Bay and Central sites, but none at Long Reef. However, an increase in the diurnal range in dissolved oxygen concentration was observed in surface waters at all three sites, and bottom water concentrations fell at the Hobsons Bay and Central sites. It seems clear that the dissolved oxygen response was most likely caused by decomposition of settling plant matter, and was not observed at the Long Reef site because there was no increase in surface chlorophyll concentration.

Further work is under way in a number of programs to improve our understanding of denitrification, and to improve confidence in the ability of this program to act as an early warning of detrimental change in Bay health.

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Introduction

The Port Phillip Bay Environmental Management Plan (EMP; NRE 2002) identified nutrients and marine pests as the two greatest risks to Bay health, and facilitated on-going tasks to reduce these risks. In particular, the efficient processing of nitrogen inputs *via* a mechanism called benthic denitrification is critical to the maintenance of the current status of the Bay (Harris *et al*. 1996). A key task of the Bay EMP was to develop and implement a program to monitor the state of Bay nitrogen cycling, and denitrification capacity in particular. The program was to be reviewed after three years.

This is the third annual report describing the progress of monitoring. The first report covered initial implementation of the monitoring over the period August-October 2002 (Longmore 2003). The second report covered the first full year of monitoring, from August 2002- July 2003. This report covers the entire period August 2002-June 2005.

Objectives

The monitoring program has the objective "To detect, as early as possible with current scientific understanding, detrimental changes to critical elements of Bay nitrogen cycling processes that indicate an increased risk of eutrophication at Bay-wide and regional scales". Eutrophication describes the excessive growth of plants that occurs in systems enriched with nutrients.

Material and Methods

The rationale for selection of sites and characteristics to be monitored was developed from recommendations of the CSIRO Port Phillip Bay Environmental Study (Newell and Harris 1997), and a subsequent workshop (Longmore 2000). The monitoring is summarized here, while sampling techniques, monitoring equipment used, chemical analysis and statistical methods were fully described in the first report (Longmore 2003).

Monitoring consists of three parts:

1. Spring and autumn monitoring of nutrient fluxes and benthic denitrification efficiency at Central and Hobsons Bay sites;
2. Continuous monitoring of key water quality characteristics at two depths at Central, Hobsons Bay and Long Reef sites;
3. Additional analysis of samples collected by the EPA Fixed Sites monitoring program for dissolved organic nitrogen concentration at Central, Hobsons Bay and Long Reef sites.

These three sites and two others (Fig 1) are sampled routinely by EPA for a number of physicochemical characteristics.

The continuous monitoring is carried out by a set of six sensor systems. The near-surface sensors are based on a Seabird SBE 19 + conductivity/temperature/depth system, with additional dissolved oxygen, fluorescence and PAR sensors. The conductivity, temperature, fluorescence and dissolved oxygen sensors are all contained within a narrow tube, treated with anti-fouling compounds to reduce biological growth. New water is pumped into the tube just prior to sampling; between samples the water in the tube surrounding the sensors becomes enriched in anti-fouling compounds. This has proven very effective, and even after two months the corrections needed to match sensor readings to calibration samples are very small. In contrast to the Seabird system, the near-bottom sensors are based on an Applied Microsystems Limited CTD 12plus system, in which all sensors are exposed to fouling. Large changes to sensor sensitivity have been observed (particularly for salinity and fluorescence) within a month of deployment. This has necessitated often large corrections to the data (see Longmore 2004 for more details).

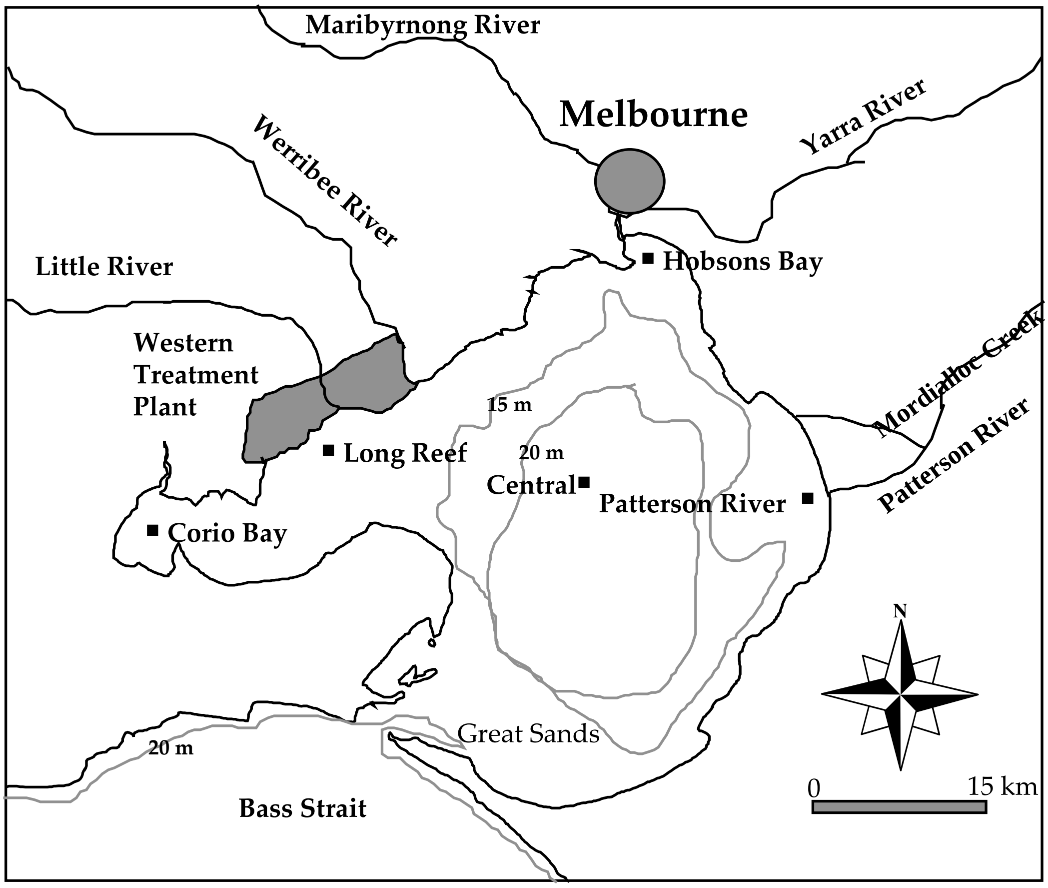

Figure 1. Sampling sites in Port Phillip Bay for both EMP and EPA monitoring.

Results

Benthic nutrient fluxes and denitrification efficiency

Benthic fluxes were measured at Central and Hobsons Bay sites in October 2002, March and October2003, March and October 2004 and March 2005 by deployments of 2-4 replicate automated benthic chambers (Table 1 and Appendix 1 Figs A1-1 to -16). Positive fluxes indicate movement from sediment to water column; negative fluxes indicate the converse. Denitrification efficiency has been chosen as the key indicator of the nitrogen cycling process, and is defined as the proportion of respired nitrogen that is recycled as N_2, compared to the bio-available nitrate, nitrite and ammonium:

D.E. =100 x N_2 flux/(N_2 flux + NH_4 flux + NO_2 flux + NO_3 flux).

Denitrification efficiency may vary from 0 to 100%. A high denitrification efficiency indicates high nitrogen recycling efficiency, and high capacity to convert nitrogen inputs to N_2 gas, which is lost to the atmosphere. A low efficiency indicates the recycling of nitrogen into forms readily available for further plant growth, and may therefore indicate susceptibility to the effects of nutrient enrichment (eutrophication). A healthy Bay will be indicated by high and stable denitrification efficiency. Following a statistical analysis of existing data, Longmore and Gason (2001) indicated that a bay-wide decline in denitrification efficiency of 30% would be a cause for alarm. Though they were able to find statistically significant seasonal differences in data collected from Port Phillip Bay in 1994-96 for the Port Phillip Bay Environmental Study, Longmore and Gason (2001) recommended that the new measurements of benthic fluxes be analysed by a simple one-way analysis of variance with sampling year, until such time as sufficient seasonal data was available to justify more detailed analysis.

Table 1. Benthic flux measurements in Port Phillip Bay, Spring 2002-Autumn 2005.
All fluxes are in m mol m^{-2} d^{-1} ± 1 S.E., except denitrification efficiency (%).

Central site	**Spring 2002**	**Autumn 2003**	**Spring 2003**	**Autumn 2004**	**Spring 2004**	**Autumn 2005**
No. observations	2	2	4	4	4	4
Dissolved oxygen	-15.3 ± 1.4	-21.0 ± 1.9	-16.3 ± 1.7	-11.6 ± 6.8	-14.5 ± 2.1	-17.2 ± 3.3
Alkalinity	11.0 ± 1.3	-	5.7 ± 1.5	1.7 ± 1.4	7.6 ± 1.0	3.4 ± 1.5
Carbon dioxide	21.8 ± 2.4	-	12.8 ± 4.3	13.5 ± 3.2	16.0 ± 1.9	12.5 ± 1.8
Ammonium	0.33 ± 0.09	0.19 ± 0.05	0.00 ± 0.03	0.37 ± 0.17	0.15 ± 0.07	0.30 ± 0.11
Nitrite	0.01 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.04 ± 0.01	0.01 ± 0.00	0.03 ± 0.00
Nitrate	0.03 ± 0.02	0.11 ± 0.10	0.00 ± 0.02	0.36 ± 0.06	0.07 ± 0.01	0.38 ± 0.03
Phosphate	0.04 ± 0.01	0.00 ± 0.11	0.06 ± 0.01	0.12 ± 0.03	0.09 ± 0.03	0.12 ± 0.01
Silicate	2.3 ± 0.2	3.1 ± 1.1	1.7 ± 0.3	3.8 ± 0.2	1.4 ± 0.1	2.8 ± 0.2
N_2	4.91 ± 0.49	6.2 ± 4.5	0.89 ± 0.59	2.08 ± 1.06	2.50 ± 0.61	1.97 ± 0.83
Denitrification efficiency	90.9 ± 4.5	88.6 ± 10.0	93.3 ± 10.2	65.9 ± 7.3	90.2 ± 4.6	67.2 ± 8.2
Hobsons Bay						
No. observations	2	4	4	4	3	4
Dissolved oxygen	-33.4 ± 5.5	-41.8 ± 2.2	-47.9 ± 4.9	-41.6 ±4.5	-27.4 ±4.5	-45.8 ± 2.3
Alkalinity	10.1 ±1.8	-	19.2 ± 2.5	25.6 ± 1.3	14.4 ± 4.9	12.3 ± 2.2
Carbon dioxide	34.5 ± 8.1	-	46.8 ± 1.0	54.8 ± 4.0	35.8 ± 7.4	32.6 ± 3.7
Ammonium	0.49 ± 0.27	1.70 ± 0.18	1.66 ± 0.30	3.44 ± 0.55	1.62 ± 0.46	2.97 ± 0.87
Nitrite	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.00	0.06 ± 0.00	0.04 ± 0.01	0.04 ± 0.01
Nitrate	0.24 ± 0.08	0.24 ± 0.03	0.24 ± 0.03	0.19 ± 0.02	0.35 ± 0.03	0.26 ± 0.09
Phosphate	0.27 ± 0.03	0.34 ± 0.03	0.25 ± 0.07	0.42 ± 0.09	0.29 ± 0.15	0.33 ± 0.03
Silicate	6.2 ± 1.6	10.9 ±0.6	10.6 ± 1.5	15.6 ± 2.6	5.3 ± 1.5	10.0 ± 0.8
N_2	-*	4.20 ± 0.31	1.59 ± 0.27	2.90 ± 0.90	3.26 ± 1.08	1.47 ± 0.20
Denitrification efficiency	84.9 ± 6.2	64.2 ± 11.2	46.1 ± 0.9	42.5 ± 8.4	57.3 ± 10.5	33.7 ± 6.3

Note: Alkalinity samples from Autumn 2003 were accidentally destroyed before analysis, so carbon dioxide flux could not be calculated. * In the absence of N_2 analyses, denitrification efficiency calculated from carbon dioxide, ammonium and nitrate fluxes.

Data were transformed, if necessary, to approximate normal distributions, before analysis of variance. The General Linear Models (GLM) procedure in the SAS statistical package was used to assign variance between differences in site and sampling period (year). The GLM procedure was used because it deals with unbalanced data, in which there are different numbers of observations for different classes (such as site, year or season). T-values were calculated for least-square means to determine the statistical significance of differences in pairs of measurements (different years). The analysis was extended to incorporate all of the benthic flux measurements carried out at the two sites since 1994, with the exception that analyses from summer 1994 and spring 2002 from both sites, and autumn 2003 from the central site, were excluded because there were less than three replicates.

Differences between sites was highly significant ($p<0.0001$) for all fluxes, and for denitrification efficiency. Fluxes of oxygen, carbon dioxide, ammonium, phosphate, and silicate were always higher, and denitrification efficiency lower, in Hobsons Bay than in the centre of Port Phillip Bay. Given these statistically significant differences, analyses were repeated for each site separately, with sample year used as a classification variable.

At the Hobsons Bay site, all fluxes, excluding dissolved oxygen, ammonium and denitrification efficiency, varied significantly between years (Table 2). None of the differences were consistent over sampling periods (i.e. there were no trends over the whole data set). Rather, the differences were usually due to a specific year. For example, phosphate and silicate fluxes from 1994 were significantly different to all subsequent measurements, while carbon dioxide fluxes in 1995 were significantly different to fluxes in 1996 and 2003-05. Denitrification efficiency was lower in autumn 2003, 2004 and 2005 than in the previous spring, but the differences between years were not statistically significant.

At the central Port Phillip Bay site, all fluxes except dissolved oxygen and carbon dioxide varied significantly with sample year. As with the Hobsons Bay site, differences were not part of a consistent trend over time.

Denitrification efficiency was lower in autumn 2003, 2004 and 2005 than in the previous spring, with the differences particularly marked for the central bay site in 2004 and 2005. It was also lower in autumn 2004 and 2005 than in previous years, but recovered to former levels in the intervening spring 2004. As a consequence, fluxes in 2005 were not different statistically from those in 1995, 1996, 2003 or 2004, but they were significantly different to those in 1994.

Table 2. Analysis of variance of benthic fluxes from Hobsons Bay and central Port Phillip Bay by site and year, 1994-2005.

Flux	Type III SS	F	Pr>F	Significant differences (s.d) ($p<0.05$) between years
Hobsons Bay				
Dissolved oxygen	1.415	1.96	0.112	-
Carbon dioxide	5.795	4.22	**0.0038**	1995 s.d. 1996-2005
Ammonium	5.228	2.33	0.0601	-
Nitrite + nitrate	0.859	10.04	**<0.0001**	1994-96 s.d. 2002-05
Phosphate	0.0298	3.01	**0.0211**	1994 s.d. 1996-2005; 1995 s.d. 1996, 2004
Silicate	22.47	6.29	**0.0002**	1994 s.d. 1996-2005; 1995 s.d. 1996-2005
DIN	3.38	2.56	**0.0421**	1995 s.d. 1996, 2004, 2005
Denitrification efficiency	0.0059	0.89	0.4983	-
Central site				
Dissolved oxygen	0.9075	2.30	0.0704	-
Carbon dioxide	0.3138	1.21	0.3289	-
Ammonium	0.9780	3.25	**0.0179**	1994, 2003 s.d. 1995, 1996, 2004, 2005
Nitrite + nitrate	1.0637	15.32	**<0.0001**	1994, 2005 s.d. rest
Phosphate	0.0050	4.61	**0.0029**	1994 s.d. 1995, 1996, 2004, 2005; 2003 s.d. 2005
Silicate	5.4096	7.44	**0.0001**	1994 s.d. rest; 1995 s.d. 2003; 2003 s.d. 2005
DIN	1.952	12.22	**<0.0001**	1994, 2003 s.d. rest
Denitrification efficiency	0.0023	4.18	**0.0051**	1994 s.d. 1995, 1996, 2004, 2005

DIN is the sum of ammonium, nitrite and nitrate. Degrees of freedom = 5; Type III SS = Type III sum of squares; F=F value; Pr>F =probability of difference exceeding F by chance (0.05 chosen as minimum level of significance). Significant results are shown in bold. Significant differences were determined by Least Squares Means t-test in pairwise comparisons between years ($p<0.05$).

Continuous water column monitoring

Characteristics measured include salinity, temperature, depth, dissolved oxygen, chlorophyll fluorescence and photosynthetically active radiation (PAR) at two depths at three sites. Data from each set of sensors is displayed (Appendix 2, Figs A2-1 to -12). Missing data indicates either that no data was collected, or that instrument malfunction or biological fouling led to unusable data. Specific causes of missing data included programming faults, battery failure, logger mounting failure, downtime while navigational piles were replaced, and programmed maintenance. For the first year of deployment, equipment was serviced in the field, to maximise the amount of data collected. However, fouling and other unforseen problems were so frequent that after the first year, the instruments were returned to the lab for extensive cleaning and recalibration. This led to the loss of at least two days of data every two months. Most recently, the temperature sensor on the bottom logger in Hobsons Bay failed in March 2005, and has not been replaced.

Temperature measurements at each site (Figs A1, 5, 9) varied in a similar seasonal manner, though the variation was smoothest at the central site. There was considerable short-term variation, even in 10 m-deep water in Hobsons Bay, presumably related to the passage of weather fronts. Thermal stratification of up to 2.5 °C was found in Hobsons Bay, with differences at the other sites of less than 0.5 °C.

Salinity at each site followed a seasonal pattern, with peaks in autumn and troughs in spring (Figs A2, 6, 10). However, the largest changes followed increased rains in spring-summer 2004-2005, which led to a sustained reduction in salinity. Salinity in bottom waters varied by about 2 (salinity has no units) at each site over the period 2002-2005. Freshwater inputs in Hobsons Bay usually affected the surface layer only (Fig 6). Salinity in the surface layer was often depressed for short periods (~ 1 day); higher salinity was presumably restored by mixing deeper into the water column. The water column in Hobsons Bay was often salinity-stratified, with salinity differences between surface and bottom waters of 1-5. The short-term impact of freshwater inputs was much less by the time water drifted to the central Bay site (Fig 2), and salinity stratification of the water column was much less frequent there. The relatively large declines in salinity at the central site surface, following heavy rains in November 2004 and February 2005, are discussed below. Because of the shallow nature of the site, surface and bottom salinity at Long Reef were often similar (Fig 10), and there is much less evidence of impact from freshwater.

Chlorophyll *a* concentration (Figs A3, 7, 11) varied rapidly over short periods (2-10 days). In general, surface and bottom chlorophyll concentrations varied over a similar range, but there were occasions, particularly at the Long reef site, when surface chlorophyll concentration was higher than bottom. At the Hobsons Bay site, this pattern could be attributed to enhanced algal growth in the usually fresher surface layer, but the peaks at Long Reef (e.g. in August 2003, May and September 2004) did not coincide with salinity stratification. The question of why such signals were not also observed by the bottom sensor, especially at such a shallow site, is unresolved. On the other hand, particularly at the Long Reef and Hobsons Bay sites, enhanced chlorophyll concentrations at the bottom may indicate resuspended benthic plants (microphytobenthos) rather than enhanced growth of phytoplankton. The response to the February 2005 flood varied at each site, and is discussed further below.

Dissolved oxygen concentration averaged 95-100% in near-surface waters, and 90-95% in bottom waters (Figs A4, 8, 12). At Hobsons Bay and central Bay sites, bottom DO concentration was often substantially below surface concentrations, indicating oxygen consumption exceeding supply. Conversely, there were occasions at all sites in which surface DO concentrations were super-saturated (>100%). Most, but not all, of the peaks in surface DO concentration coincided with peaks in chlorophyll concentration, indicating active production by plants. There were occasions when bottom DO concentrations also exceeded saturation, and in general these also coincided with peaks in bottom-water chlorophyll. On the other hand, peaks in bottom-water chlorophyll did not always lead to peaks in DO; often they were accompanied by declines. Processes which resuspend the sediments (e.g. strong winds) may cause this pattern (high chlorophyll and low DO). The response of DO to the February 2005 flood is discussed below.

Dissolved organic nitrogen monitoring at EPA sites

Samples were collected by EPA for particulate nitrogen analysis on 10 occasions during 2002-05. The purpose of collecting these samples is to allow us to calculate dissolved organic nitrogen concentration. A healthy Bay may be indicated by stable and low concentrations. Dissolved organic nitrogen median concentrations in 2002-2005 were lower than the 1990-95 median at all sites (Table 3).

Table 3. Particulate and dissolved organic nitrogen concentrations in 2002-2005, compared to historic data.

Site	Particulate N		Dissolved organic N	
	2002-05 (n= 10)	1990-95 (n=130)	2002-05 (n= 10)	1990-95 (n=130)
Central	1.45-4.26 (2.0)	0.6-2.5 (1.6)	6.38-10.39 (7.6)	6.7-12.5 (9.7)
Hobsons Bay	1.40-4.48 (3.1)	0.14-8.82 (3.4)	6.68-11.0 (8.8)	7.4-18.7 (11.0)
Long Reef	1.14-6.74 (3.1)	0.52-8.43 (2.8)	9.17-13.91 (11.0)	7.9-19.4 (11.4)

Concentrations in μM, shown as range and median. Historic data drawn from Longmore *et al.* 1996.

Discussion

Benthic nutrient fluxes and denitrification efficiency

All of the fluxes were within the ranges previously measured in central Port Phillip Bay and Hobsons Bay (Table 4). The data indicate generally higher fluxes at both sites in autumn than in spring, and much higher fluxes in Hobsons Bay in both seasons than in central Port Phillip Bay. Both of these observations also accord with past work, and reflect the generally higher supply of organic matter to the sediments in Hobsons Bay. The key indicator, denitrification efficiency, was always higher in central Port Phillip Bay than in Hobsons Bay. In Hobsons Bay in spring 2002, denitrification efficiency was higher than the historic median, which could be attributed to a lower than normal organic supply, due to low riverine inputs. However, carbon dioxide fluxes in later samples were closer to previous measurements, as was denitrification efficiency. There is some evidence of a fall in denitrification efficiency in autumn 2004 and autumn 2005 at both sites, compared to the previous spring.

Table 4. Historical benthic flux measurements in Port Phillip Bay (1993-95).

Flux	Central	Hobsons Bay
N. obs	18	25
Dissolved oxygen	-12 to –39 (-21)	-14 to –86 (-46)
Carbon dioxide	6-30 (15)	20-120 (30)
Ammonium	0-0.8 (0.12)	0-4.0 (0.95)
Oxidised N	0-0.4 (0.16)	0-0.7 (0.12)
Phosphate	0-0.17 (0.08)	0-0.44 (0.13)
Silicate	0.4-3.8 (2.7)	0-15 (2.8)
Denitrification efficiency	61-100 (85)	14-100 (65)

All fluxes in m mol m^{-2} d^{-1}, except denitrification efficiency (%). Range and median shown.

Denitrification efficiency could fall for two reasons: a failure in nitrification, or a failure of denitrification. Nitrification could fail if oxygen concentrations in the sediments became limiting (too low), while denitrification could fail if oxygen concentrations in the sediment were too high, or if the mechanism(s) by which nitrate is transported from oxygen-rich to oxygen-poor zones in the sediment failed. Higher nitrate fluxes accompanied the decline in denitrication efficiency in central PPB in autumn 2004 and 2005 compared to the previous spring, indicating the partial failure of denitrification, rather than of nitrification. Even so, oxygen and carbon dioxide fluxes were not particularly high, suggesting that a decline in the transport of water through the sediment is a more likely explanation for decline in denitrification efficiency (e.g. from a change in fauna in and on the sediment). The weakness in this argument is that the declines in denitrification appeared to be seasonal (in autumn), and there was no evidence of an impact in spring. In spring 2003 and 2004, denitrification efficiency at the central site was higher than the historic median (Table 4).

Such variations could also arise from increasing heterogeneity in the sediment, so that they are not seasonal variations, but rather random “noise”. Recent studies (Dr Jeff Ross, unpublished) have indicated that the impact of *Sabella* on denitrification depends on how the *Sabella* are distributed: clumps have a different impact to the same number of animals more evenly distributed. Presumably the same may apply to the many infauna in the Bay whose role in denitrification remains unknown. The power analysis on which benthic flux sampling effort is based (Longmore and Gason 2001) assumes that the distribution of fauna which play a role in denitrification does not change over time. Increasing variability in fauna (if it exists) means to the power to detect a specific level of change at current sampling effort will be lower than expected. Because the changes do not appear to be part of a consistent longer-term pattern, they are not yet regarded as cause for concern.

Continuous water column monitoring

After correction, the data were summarised statistically (Table 5) for comparison with State Environment Protection Policy (SEPP) objectives for Port Phillip Bay (Government of Victoria 1997). Dissolved oxygen in surface waters at Central and Hobsons Bay sites met the objective almost completely, but only bottom waters from the Central site complied completely. Almost a quarter of the observations from bottom waters in Hobsons Bay were below 90% saturation. The dissolved oxygen concentrations at Long Reef were somewhat perplexing: lowest, median and 90th percentiles were lower in surface water than in bottom water. It could be argued that the large diurnal range found in surface waters, and the high maximum DO concentration, were responses to high plant biomass; lower median DO in surface waters would then reflect the greater respiration at night due to the higher biomass. Given that the two depths are only 2 m apart, and that there was no large difference in chlorophyll concentration between depths at this site, this explanation is not convincing. However, whatever the reason for the differences, these observations indicate the value of long-term data sets. The conclusion reached after the first 12 months of data was that the relatively low bottom DO observed in Hobsons Bay confirmed the conclusion reached during the Port Phillip Bay Environmental Study, that Hobsons Bay is the area most under stress. After 30-36 months of data, the large diurnal excursions in DO concentration indicate that the Werribee coast is also under stress.

Table 5. Summary statistics for continuous measurements

Site	Salinity	Dissolved oxygen (% sat)	Chlorophyll *a* (μg L^{-1})
Central surface	N. obs =14,211 Min = 34.60 Median = 36.43 90 %ile = 37.07 Max = 37.24	N. obs = 14,414 Min = 79.1 Median = 97.7 90 %ile = 101.0 Max = 117.1 2% of observations < 90% sat	N. obs =14,240 Min = 0.07 Median = 0.71 (1.0) 90 %ile = 1.30 (2.0) Max = 5.97
Central bottom	N. obs =19,333 Min = 35.54 Median = 36.58 90 %ile = 37.15 Max = 37.56	N. obs = 20,251 Min = 76.2 Median = 95.7 90 %ile = 99.7 Max = 115.0 15% of observations < 90% sat	N. obs = 14,600 Min = 0.03 Median = 0.81 (1.0) 90 %ile = 1.56 (2.0) Max = 2.93
Hobsons Bay surface	N. obs =17,714 Min = 26.96 Median = 36.11 90 %ile = 36.84 Max = 37.30	N. obs = 17,714 Min = 81.6 Median = 97.7 90 %ile = 101.7 Max = 140.9 3% of observations < 90% sat	N. obs =17,036 Min = 0.28 Median = 2.30 (2.5) 90 %ile = 4.20 (4.0) Max = 10.8
Hobsons Bay bottom	N. obs =21,914 Min = 33.30 Median = 36.63 90 %ile = 37.30 Max = 38.29	N. obs =20,871 Min = 63.7 Median = 95.0 90 %ile = 101.8 Max = 115.5 24% of observations < 90% sat	N. obs =21,052 Min = 0.21 Median = 2.17 (2.5) 90 %ile = 4.27 (4.0) Max = 25.9
Long Reef surface	N. obs =16,271 Min = 35.22 Median = 36.55 90 %ile = 37.36 Max = 38.56	N. obs =14.517 Min = 60.5 Median = 93.6 90 %ile = 102.3 Max = 148.0 31% of observations < 90% sat	N. obs =15,299 Min = 0.20 Median = 0.78 (2.5) 90 %ile = 1.48 (4.0) Max = 5.41
Long Reef bottom	N. obs = 21,705 Min = 33.76 Median = 36.66 90 %ile = 37.44 Max = 38.34	N. obs =21,600 Min = 77.8 Median = 98.7 90 %ile = 106.2 Max = 127.4 4% of observations < 90% sat	N. obs =20,205 Min = 0.10 Median = 0.81 (2.5) 90 %ile = 1.61 (4.0) Max = 9.86

SEPP chlorophyll objectives shown in brackets. SEPP dissolved oxygen objective is > 90% saturation in surface waters, and 1 m from the bottom, except in the General segment, where annual 90th percentile should be >90% saturation.

Fiftieth percentile compliance with SEPP for chlorophyll *a* was complete for all sites, at both depths. Ninetieth percentile compliance was met at both depths at the Central and Long Reef sites, but failed in surface and bottom waters at the Hobsons Bay site. Given the possible errors in converting fluorescence to chlorophyll *a* concentrations (see Longmore 2003), and the small margin by which compliance failed, the apparent failure is not an immediate cause for concern.

Median salinity at each site exceeded the maximums observed at West Channel Pile (36.21) and Fawkner Beacon (35.52) during continuous monitoring over the period September 1990-July 1999 (Longmore, unpublished), indicating that the Bay has been more saline in 2002-2005 than in the previous decade. This is not surprising, given that the annual rainfall in the Port Phillip Bay catchment has been below average for eight of the past ten years.

According to the PPBES model, a 30% decline baywide in denitrification efficiency would be reflected by a 28% increase in chlorophyll *a* concentration (Longmore and Gason 2001). No long-term statistical change has been detected for either indicator in the first three years of this program. At most, denitrification efficiency declined by ~ 20% at the Central site over 2004-05, and apart from the response to the flood in February 2005, chlorophyll *a* concentration has not increased over the period 2002-2005.

Response to the February 2005 flood

The continuous data were examined in greater detail, to seek responses at each site to the February 2005 flood in the Yarra and Werribee Rivers (Appendix 3, Figs A3-1 to -6).

Chlorophyll *a* concentration increased for about 10 days after the flood in Hobsons Bay, with a greater increase in bottom waters than in surface waters. This could be either a response to the settling of riverine plant material, or resuspension of MPB from the sediment. A secondary peak was observed in surface waters 4-5 weeks after the flood. Dissolved oxygen concentrations responded immediately to the flood, with an increase to supersaturation in surface waters, and a decline to below 70% in bottom waters. Concentrations recovered to above 80% within a month The diurnal range in surface DO concentrations reached 30% following the flood, and fell to less than 10% within a month.

At the Central site, surface chlorophyll concentration increased initially to ~ 3 $\mu g\ L^{-1}$ at the surface, and gradually declined over the next six weeks, while bottom concentrations gradually increased, and remained above 1.5 $\mu g\ L^{-1}$ until May 2005. A second, much larger response (to 6 $\mu g\ L^{-1}$) was observed in surface waters six weeks after the flood, but persisted for only three days. Dissolved oxygen concentration responded immediately to the increased biomass following the flood, leading to supersaturation in surface waters. The diurnal range also increased at the surface to ~ 10%. There was no diurnal signal at the bottom, but concentration fell to less than 80% for about a week following the flood, then gradually recovered over the next month to close to surface concentrations.

The flood caused no response in the surface chlorophyll sensor at Long Reef, but there was a significant short-term (3 day) increase at the bottom. Since the bottom chlorophyll sensor is only ~ 10 cm above the sediment, this response is likely to have been caused by resuspended sediment. Salinity declined coincident with the flood, so it cannot be argued that the Long Reef site was not affected by a plume. Plant biomass along the Werribee coast is dominated by macroalgae, and it is possible that any response to the flood may have been restricted to the macroalgae. The most apparent impact on dissolved oxygen concentration was in surface waters, with the diurnal range reaching ~ 40% and persisting for the next six weeks. The small diurnal range in bottom waters did not change, nor did the absolute level, in response to the flood.

The responses to the flood therefore varied between sites, with short-lived responses in surface water chlorophyll at Hobsons Bay and Central sites, but none at Long Reef. However, an increase in the diurnal range in dissolved oxygen concentration was observed in surface waters at all three sites, and bottom water concentrations fell at the Hobsons Bay and Central sites. It seems clear that the dissolved oxygen response was most likely caused by decomposition of settling plant matter, and was not observed at the Long Reef site because there was no increase in surface chlorophyll concentration.

It would be possible to reach an understanding of the significance of the flood, and the Bay's response to it, if we knew how much nitrogen was carried into Port Phillip Bay by the flood. This information is not yet available.

Dissolved organic nitrogen monitoring at EPA sites

Too few samples have been collected so far to draw statistically robust conclusions about any trends in dissolved organic N concentration, or how sensitive it is as an indicator. Qualitative analysis indicated that dissolved organic nitrogen concentrations were all within the historical range, and the median was slightly below the 1990-97 median at all three sites. It should be noted that sampling by EPA has been much less intensive than expected; for example, samples were collected only four times in 2004-05. Any large gaps in the sampling schedule make it less likely that we will be able to statistically detect any change in nitrogen cycling in the Bay.

Other issues

Strengths and weaknesses of the existing continuous monitoring system

Electronic recording of physicochemical data is both a strength and a weakness. Despite the problems with fouling of the near-bottom sensors, which was fully discussed in Longmore (2004), an extremely large amount of data has already been recovered. Data recovery from the bottom sensors in the 23,952 hour period over which sampling extended (August 2002-May 2005) varied between 84% and 91%. Data recovery from the surface sensors (March 2003 – May 2005) varied between 75-93%. Overall recovery was 83%. The volume of data collected already exceeds all previous measurements of salinity, dissolved oxygen and chlorophyll fluorescence in Port Phillip Bay.

Areas of uncertainty

In the first report, areas were identified which could lead to uncertainty as to whether or not the current approach will correctly provide an early warning of change. The table from the first report is reproduced here (Table 6) and has been updated as appropriate. In particular, the almost completed ARC Linkage project, and studies carried out for the Channel Deepening EES and Supplementary EES, will contribute significantly to our understanding of nutrient cycling in the Bay, and vulnerability to impact from external factors.

Table 6. Areas of residual uncertainty surrounding the early warning of change

Uncertainty	Current understanding	Risk of ignoring uncertainty	Possible remedy
Appropriate indicators; discussed extensively in Longmore (2000).	We are monitoring all five of the changes we expect to see arising from increased nutrient input; statistical analysis indicates denitrification efficiency is the most sensitive	1. Change may occur, but not in the indicators we use; probably low risk. 2. Fluorescence affected by fouling, leading to false positive responses or failure to detect change	1. Needs further study 2. Develop automated cleaning process; replace with less vulnerable sensors; reconsider remote sensing
Timing of change; discussed extensively in Longmore (2000).	1. Continuous water column monitoring should capture even brief changes. 2. Sediments integrate water column production over time; current sampling aims to capture spring and summer peaks in plankton production	1. Little risk 2. Not clear how long the integration applies to; sampling intervals may be too great to detect change; changes may occur outside the sampling period	1. None required 2. Needs further study
Location of change; discussed extensively in Longmore (2000).	Expect change to be greatest near the known major nutrient inputs	Changes, especially on local scales, may not be detected	Needs further study (NHT funding sought); CSIRO model output may be useful.
Importance of infauna/exotics	Bio-irrigation greatly enhances denitrification efficiency; invasion by exotics may lead to unpredictable changes	Changes occur unrelated to nutrient inputs	ARC Linkage project addressing this is within one year of completion
Other nutrient supplies/losses	1. Channel dredging may impact on local scales; 2. Aquaculture may impact on local scales; 3. Burial in sediments 4. Nutrients enhance macroalgae, rather than plankton	1. Changes occur unrelated to monitored nutrient inputs. 2. "Offsets" may not be effective 3. May create a store for future potential massive release 4. Macroalgae not monitored	1. SEES underway will address this; 2. Allow margins for error 3. Evaluate through pilot study; SEES studies may also assist 4. Pilot study suggested

Developing techniques for early detection of impact

The direct measurement of denitrification depends on the measurement of a small change (1-2 µM) in dissolved N_2 concentration against a high background (400-500 µM). Only one laboratory in Australia offers this analysis on a commercial basis, and the turn-around for samples is up to two months. With such a long delay between sampling and results, the current method of denitrification monitoring offers little as an early warning of impending impact. Rather, it offers some measure of early detection of an impact that has already occurred. An alternative method is currently under development which may offer a much more rapid detection of impact, which in turn may allow earlier intervention if necessary. The alternative method depends on the strong relationships which have been observed in Port Phillip Bay between respiration rate, ammonium flux and denitrification (Fig 2). The relationships are based on the following rationale.

At low carbon supply rates, respiration rates are low, nitrogen regeneration is low, oxygen concentration in the sediment remains high, so that nitrification is effective, but sufficient anoxic zones exist to allow denitrification to proceed. Ammonium, nitrate and N_2 fluxes are low, and denitrification efficiency is high. As organic supply increases, respiration and N flux increase, and though denitrification increases, the proportion recycled as N_2 (denitrification efficiency) declines. At higher respiration rates, oxygen becomes limiting in the sediments. Nitrification is then suppressed, so that denitrification is also limited. N is increasingly recycled as ammonium. If these relationships are sufficiently robust, measurement of ammonium and respiration fluxes may be used to predict denitrification efficiency. The advantage of this approach is that respiration and ammonium flux can be measured *in situ* electronically, so that turn-around of information is extremely fast (within 24 hours). This technique is being developed and tested for the Port Phillip Bay Channel Deepening Project, and could be applied to the PPB EMP program if it is successful. Much greater replication would be possible for the same effort currently dedicated to direct measurement of denitrification.

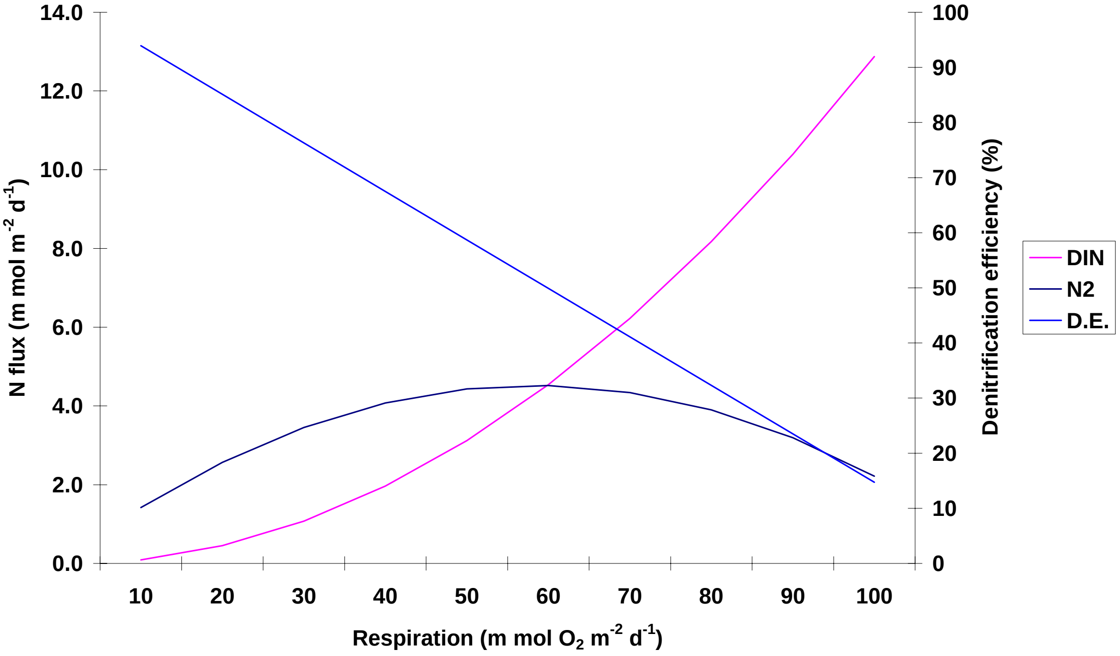

Figure 2. Variation of denitrification efficiency (D.E.), ammonium (DIN) and N_2 flux with respiration in northern Port Phillip Bay. Derived from measurements in Hobsons Bay and central Port Phillip Bay, 1994-2005.

Conclusions

Benthic nutrient fluxes during 2003-2005 were low in central Port Phillip Bay and denitrification efficiencies high. Fluxes in Hobsons Bay were 2-3 times those in central Bay, and though denitrification efficiency dropped from exceptionally high levels in Hobsons Bay in spring 2002 and autumn 2003 to lower levels in autumn 2004 and 2005, it was still well within the range of previous observations. The changes do not appear to be part of a longer-term decline in Bay health.

Continuous monitoring of bottom waters has been carried out at three sites since August 2002, and surface waters since March 2003. Data has been lost from biological fouling of bottom sensors, but not from surface sensors. Some data has also been lost due to battery failure and programming errors. The differences in susceptibility to fouling of the two different types of instrument and resultant differences in data reliability are so pronounced that the project should consider how we can replace the bottom sensors as soon as possible. At July 2005 exchange rates, replacement cost is approximately $120, 000. Because of the fouling, data processing takes much longer than expected.

The Bay has been saltier than at any time since 1983, because of low runoff, but the rains over winter 2004 and February 2005 led to a small decline in salinity. Chlorophyll over winter 2003 was also at historically low levels. Dissolved oxygen and chlorophyll concentrations complied almost completely with SEPP objectives, though bottom oxygen concentrations fell below the SEPP objective for a substantial number of samples at Hobsons Bay and Long Reef sites.

The responses to the February 2005 flood varied between sites, with short-lived responses in surface water chlorophyll at Hobsons Bay and Central sites, but none at Long Reef. However, an increase in the diurnal range in dissolved oxygen concentration was observed in surface waters at all three sites, and bottom water concentrations fell at the Hobsons Bay and Central sites. It seems clear that the dissolved oxygen response was most likely caused by decomposition of settling plant matter, and was not observed at the Long Reef site because there was no increase in surface chlorophyll concentration.

If denitrification efficiency declined baywide by 20%, we would expect to see an almost equivalent increase in chlorophyll and dissolved organic nitrogen concentration. No such changes have been seen.

On the basis of the data collected so far there is nothing to indicate a baywide decline in denitrification efficiency; nor have there been changes to the other indicators (chlorophyll, dissolved oxygen, dissolved organic nitrogen concentrations) that would accompany such a change.

Data has been forwarded to the State Government DataWarehouse so that the data may be accessed by the public.

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